



Structure elucidation of cyclohexene (9Z)-octadec-9-enyl ethers isolated from the leaves of *Uvaria cherrevensis* (Annonaceae)

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ABSTRACT

The phytochemical investigation of the leaf extract of *Uvaria cherrevensis* (Annonaceae) yielded three new cyclohexene (9Z)-octadec-9-enyl ethers, cherrevenols M-O (**1–3**), and a known fatty ester derivative (**4**). The structures of the isolated compounds were elucidated by spectroscopic and computer-aided molecular modelling methods. Ozone Induced Dissociation (OzID) mass spectrometry was employed to determine the C-9 position of the side chain olefinic double bonds, while ¹³C NMR spectroscopy indicated their (Z)-configurations. All isolated compounds were evaluated for their antimalarial and cytotoxic activities; all were inactive.

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1. Introduction

Cyclohexene long-chain saturated fatty esters were isolated in earlier studies from two species of *Uvaria*, *U. dulcis* and *U. dac* [1,2]. These compounds exhibited cytotoxic activities [2]. Previous studies on *U. cherrevensis*, Annonaceae family, found various types of secondary metabolites, including alkaloids, cyclohexenes, flavonoids, naphthalene derivatives, and terpenoids [3–8]. We report here the first isolation of cyclohexene (9Z)-octadec-9-enyl ethers, cherrevenols M-O (**1–3**), from the leaf extracts of *U. cherrevensis*. The application of ¹³C NMR and Ozone Induced Dissociation (OzID) mass spectrometry were used to determine the (Z) configuration

and C-9 position of the olefinic moiety of the C-18 ether side chains, respectively. The latter would otherwise be difficult to determine because of the lack of 2D NMR correlations due to overlapping of the side chain ¹H and ¹³C NMR resonances. The isolated compounds were evaluated for their antimalarial and cytotoxic activities.

2. Results and discussion

The leaf extracts of *U. cherrevensis* were subjected to chromatographic separation techniques which led to the isolation and characterization of three new compounds, cherrevenols M-O (**1–3**), and a known compound, dulcisene A (**4**) (Fig. 1) [2].

Cherrevenol M (**1**) was obtained as a white amorphous solid. Its molecular formula, C₃₄H₅₂O₇, was deduced from its HRESIMS data, which showed an [M+Na]⁺ ion at *m/z* 595.3615 (calcd. for C₃₄H₅₂O₇Na, 595.3611). The UV spectrum exhibited a maximum absorption band at 207, 229, and 278 nm while the IR spectrum showed bands for hydroxy and carbonyl groups at 3454 and

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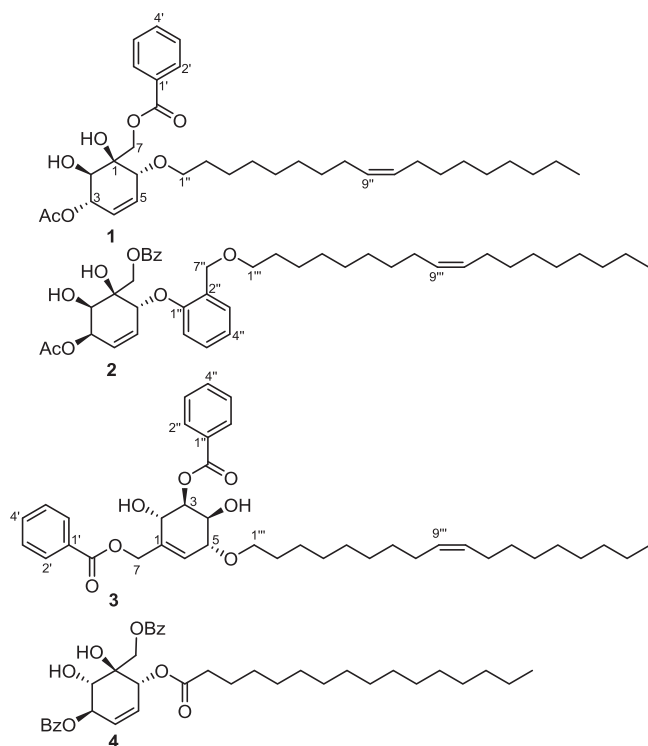


Fig. 1. The structures of isolated compounds from the leaves of *U. cherreensis*.

1722 cm⁻¹, respectively. The ¹H NMR spectroscopic data of **1** (Table 1) was very similar to that of ellipeiopsol A (Fig. 2, see Supporting Information (Table S1) for details) previously isolated from this plant [3]. The main difference was the addition of resonances for an *O*-(*Z*)-octadec-9-enyl moiety. The diastereotopic oxymethylene protons CH₂-1'' (δ_{H} 3.65 and 3.47) showed HMBC cross-peaks to C-6 (δ_{C} 75.9) and thus the 6-*O*-(*Z*)-octadec-9-enyl moiety was attached to the C-6 oxygen substituent. The position of the double bond in the C-18 side chain was determined by OzID mass spectrometry [9,10]. In this experiment, the sodiated molecular ion (m/z 595.3605) was isolated in an ion trap and exposed to ozone for 4 s. This produced the ozonide intermediate (m/z 643.3470 [$\text{M} + \text{Na} + \text{O}_3$]⁺) and its oxidative cleavage products corresponding to aldehyde (m/z 485.2146) and Criegee ions (m/z 501.2091) (Figs. 4 and 5). These results allowed us to unequivocally assign the double

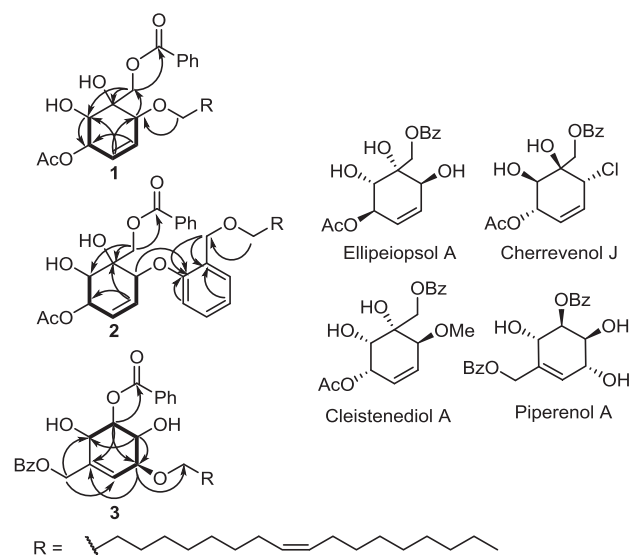


Fig. 2. Selected keys HMBC (¹H → ¹³C) and COSY (bold line) correlations for compounds **1–3** and the structures of known natural products.

bond at C-9'' [11,12]. The ¹³C NMR chemical shifts for C-8''/C-11'' (δ_{C} 27.37 and 27.35), C-9'' (δ_{C} 130.0) and C-10'' (δ_{C} 130.1) indicated that double bond had the (*Z*)-configuration [12]. The relative configuration of **1** at C-2 and C-3 was assigned from the $J_{2,3}$ value of 6.3 Hz which indicated that H-2 and H-3 had a *trans* 1,2-diaxial-like relationship [8]. The NOESY spectrum showed a correlation between the diastereotopic CH₂-7 (δ_{H} 4.79 and 4.60) resonances and the resonance for H-2 (δ_{H} 4.04) and no correlations between H-6 (δ_{H} 3.94) and CH₂-7 or between H-6 and H-2. This indicated that CH₂-7 and H-2 were on the same face of the cyclohexene ring structure, while H-6 was on the opposite face of the ring to H-2 and CH₂-7. When the proposed structure of **1** was generated computationally, [B3LYP/6-31G(d)] using the Gaussian 09 basis set. (Fig. 3), the distance between H-2 and CH₂-7 was calculated to be 2.58 Å, which correlated well with the experimental findings from the NOESY study. The specific rotation of **1** was of the same sign as that of cherrevenol J (Fig. 2), which was also isolated from this plant [8], [α]_D²⁴ –90 (c 0.1, MeOH) and [α]_D¹⁹ –84 (c 0.2, MeOH), respectively. In addition to this, the ECD spectra of these compounds exhibited similar negative Cotton effects at 207 and 203 nm, respectively. Therefore, **1** (cherrevenol M) was assigned as having the 1*R*,2*R*,3*S*,

Table 1
¹H (400 MHz) and ¹³C (125 MHz) NMR spectroscopic data of cherrevenol M (**1**) in CDCl₃.

1					
Position	δ_{H} , (J in Hz)	δ_{C} , type	Position	δ_{H} , (J in Hz)	δ_{C} , type
1		75.9 C	1''	3.65, dt (9.0, 6.4)	70.8 CH ₂
2	4.04, d (6.3)	71.5 CH		3.47, dt (9.0, 6.4)	
3	5.43, m	74.0 CH	2''	1.53, m	30.2 CH ₂
4	5.74, dd (10.2, 2.7)	126.9 CH	3''	1.26, m	26.3 CH ₂
5	6.03, ddd (10.2, 3.9, 1.8)	128.2 CH	4''-7'' and 12''-15''	1.26, m	29.92, 29.9, 29.85, 29.7, 29.6, 29.5, 29.47, 29.4 CH ₂
6	3.94, d (3.9)	75.9 CH	8''	2.00, m	27.37 ^a CH ₂
7	4.79, d (12.0)	67.1 CH ₂	9''	5.34, m	130.0 CH
	4.60, d (12.0)		10''	5.34, m	130.1 CH
1'		129.9 C	11''	2.00, m	27.35 ^a CH ₂
2',6'	8.05, dd (7.6, 1.5)	129.8 CH	16''	1.26, m	32.1 CH ₂
3',5'	7.46, t (7.6)	128.7 CH	17''	1.26, m	22.8 CH ₂
4'	7.59, tt (7.6, 1.5)	133.4 CH	18''	0.88, t (6.9)	14.3 CH ₃
3-OC(O)Me		171.9 C	7-OC(O)		167.3 C
3-OC(O)Me	2.07, s	21.3 CH ₃			

^a Exchangeable carbons.

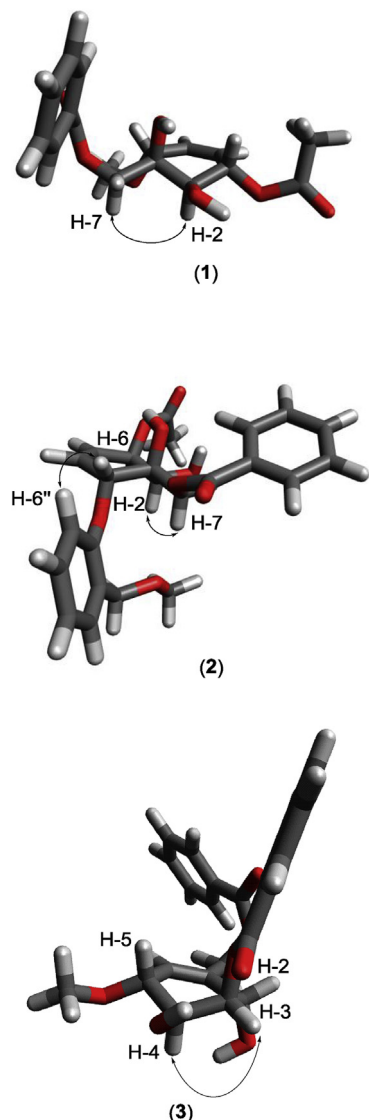


Fig. 3. Experimental NOE correlations projected onto DFT minimized molecular models [B3LYP/6-31G(d)] of **1–3** (calculated on the corresponding Me ethers).

6*R* configuration.

Cherrevenol N (**2**) was isolated as a colourless oil and showed an HRESIMS $[M + Na]^+$ ion at m/z 701.4019 (calcd for $C_{41}H_{58}O_8Na$, 701.4029). The UV spectrum showed absorption bands at 203, 230, and 275 nm and the IR spectrum showed bands for hydroxy and carbonyl functionalities at 3437 and 1723 cm^{-1} , respectively. The 1H and ^{13}C NMR spectroscopic data were similar to those of **1** except for additional resonances characteristic of a 2-hydroxyphenylmethanol moiety [δ_H 7.32 (1H, dd, $J = 7.5, 1.8$ Hz), 7.26 (1H, td, $J = 8.2, 1.8$ Hz), 6.99 (1H, td, $J = 7.5, 1.8$ Hz), 6.96 (1H, d, $J = 8.2$ Hz), 4.81, and 4.28 (each 1H, d, $J = 11.9$ Hz)] in **2**. The oxymethine proton H-6 (δ_H 4.97) showed a HMBC cross-peak to C-1'' (δ_C 157.3) while CH_2 -1''' (δ_H 3.49 and 3.46) correlated with C-7'' (δ_C 69.0) indicating that the 2-hydroxyphenylmethanol moiety was linked between C-6 of the cyclohexene ring structure and C-1''' of the (Z)-octadec-9-enyl substituent (Fig. 2). The ^{13}C NMR chemical shifts of C-8''', C-9''', C-10''' and C-11''' at δ_C 27.4, 130.0, 130.1, and 27.4, respectively were characteristic of a (Z)-olefinic configuration [12]. The OzID mass spectrum of **2** exhibited aldehyde and Criegee ions at m/z 591.2566 and 607.2517, respectively that indicated the alkene was located at C-9''' as shown in Figs. 4 and 5. The relative configuration of **2** was determined from the examination of the NOESY spectrum. Key correlations were observed between H-2 (δ_H 4.29) and CH_2 -7 (δ_H 4.87 and 4.72), and between H-6 (δ_H 4.97) and H-6'' (δ_H 6.96) which validated the optimized molecular model as the calculated distance between these protons were 2.52 Å and 2.00 Å, respectively. In contrast, H-6 showed no NOESY correlations to CH_2 -7, indicating that these protons were on the opposite face of the ring structure. In addition, the coupling constant value between H-2 and H-3 was 3.5 Hz which indicated their relative 1,2 equatorial-axial like relationship (Fig. 3). The specific rotation of **2** $[\alpha]_D^{25} -57$ (c 0.1, MeOH) was opposite in sign to that of cleistenediol A $[\alpha]_D^{20} +190$ (c 0.3, MeOH) (Fig. 2), isolated from *Cleistochlamys kirkii* [13], and so suggests an opposite configuration of the known compound. Therefore, the configuration of cherrevenol M (**2**) was assigned as 1*R*,2*R*,3*R*,6*R*.

Cherrevenol O (**3**) was isolated as a colourless oil. Its molecular formula, $C_{39}H_{54}O_7$, was deduced from its HRESIMS data, which showed an $[M + Na]^+$ ion at m/z 657.3770 (calcd for $C_{39}H_{54}O_7Na$, 657.3767). The UV spectrum exhibited adsorption bands at 234 and 274 nm, and the IR spectrum showed bands at 3423 and 1722 cm^{-1} that corresponded to hydroxy and carbonyl groups, respectively. The NMR spectroscopic data of **3** were similar to those of piperenol A (Fig. 2), isolated from *Piper cubeb* [14], except that **3** contained 1H

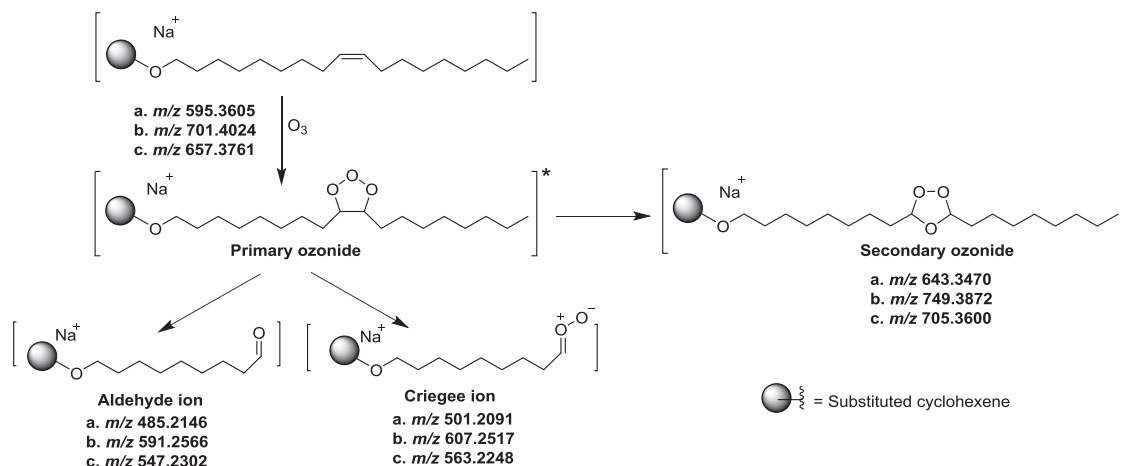


Fig. 4. Proposed mechanism for OzID fragmentation of **1–3** (a–c) [Criegee ion (representative structure shown but isomers of the carbonyl oxide cannot be excluded)].

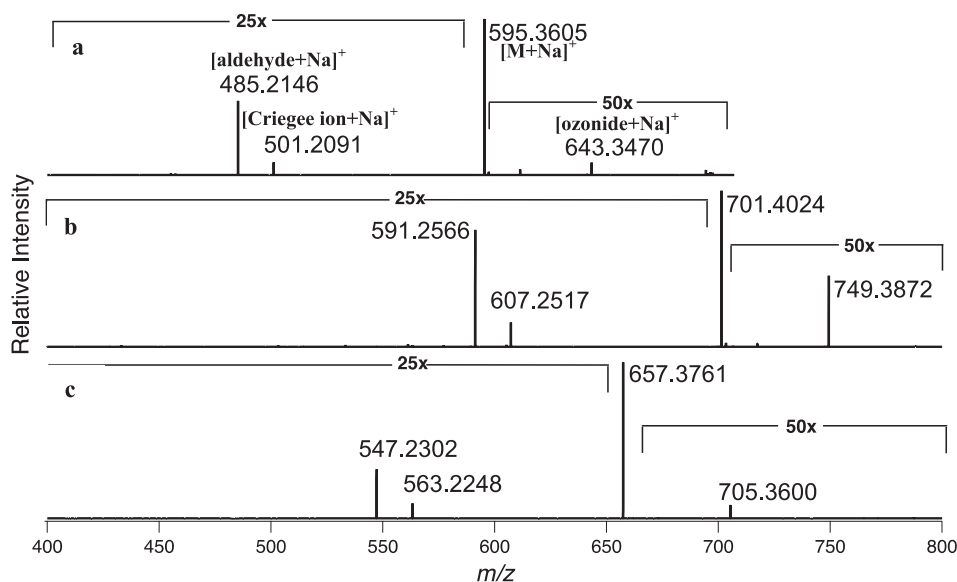


Fig. 5. The OzID spectra from sodium adducts of **1–3** (a–c).

and ^{13}C NMR resonances characteristic of a (Z)-octadec-9-enyl moiety. The oxymethine proton H-5 (δ_{H} 4.13/ δ_{C} 76.8) and the diastereotopic oxymethylene protons $\text{CH}_2\text{-1}'''$ of the octadec-9-enyl moiety (δ_{H} 3.69 and 3.63/ δ_{C} 70.4) showed HMBC cross-peaks to each other which indicated that the (Z)-octadec-9-enyl moiety was attached to the C-5 oxygen substituent of the cyclohexene ring (Fig. 2). The ^{13}C NMR chemical shifts for C-8'''/C-11''' (δ_{C} 27.4), C-9 (δ_{C} 130.0), and C-10 (δ_{C} 130.1) corresponded to the (Z)-olefinic configuration [12]. The double bond position of the long chain unit was determined by the OzID method. More specifically, the OzID spectrum showed an ion at m/z 705.3600 $[\text{M} + \text{Na} + \text{O}_3]^+$ for the corresponding ozonide. The corresponding aldehyde and Criegee ions were observed at m/z 547.2302 and 563.2248, respectively which indicated that the double bond was at C-9''' as shown in Figs. 4 and 5. The relative configuration of **3** was assigned on the basis of the $J_{2,3}$ value of 4.8 Hz and $J_{4,5}$ value of 6.5 Hz which indicated 1,2-diequatorial-like and 1,2-diaxial-like couplings, respectively [8]. The correlations between H-3 (δ_{H} 5.50) and H-4 (δ_{H} 4.31), and between H-3 and H-2 (δ_{H} 4.52) in the NOESY spectrum, were consistent with the calculated inter-proton distances of 2.52 Å and 2.54 Å, respectively from the optimized structure (Fig. 3). These J values, NOESY correlations and molecular modelling results fully supported the proposed structure of **3**. The ECD spectrum of **3** was similar to that of piperenol A [14] which exhibited negative Cotton effects at 234 and 235 nm, respectively. This in combination with the above data led to the assignment of the configuration of **3** (Cherrevenol O) as 2S,3R,4S,5R.

These polyoxygenated long-chain cyclohexene compounds (**1–4**) were examined for their antimalarial activities against *P. falciparum* strains (TM4/8.2 and K1CB1) and for cytotoxicity against human mouth epidermal carcinoma (KB cells) and normal kidney epidermal cells of African green monkey (Vero cells). All compounds were inactive ($\text{IC}_{50} > 50 \mu\text{M}$).

3. Conclusions

A phytochemical studied of the leaf extracts of *U. cherreensis* led to the isolation and characterization of three new cyclohexene (9Z)-octadec-9-enyl ethers (**1–3**), and a previously reported compound (**4**). While long chain ester derivatives are known (e.g. **4**),

this is the first report of analogous ether derivatives. The structures of these compounds were characterized using spectroscopic techniques. Ozone Induced Dissociation (OzID) mass spectrometry proved critical in determining the double bond position of the octadec-9-enyl side chain. Importantly, ozonide formation was chemoselective for the less hindered side chain double bond. This method has been successful in the determining the double bond position of natural fatty esters [15,16], but to the best of our knowledge, this is the first example of the application of OzID to naturally occurring long chain ethers.

4. Experimental section

4.1. General

Optical rotations were measured at the sodium D-line (590 nm) on a Jasco 2000 series polarimeter. Melting points were determined on a SANYO Gallenkamp melting point apparatus and are uncorrected. The UV–vis absorption spectra and circular dichroism (CD) were measured with a Jasco J-180 spectrophotometer. The infrared (IR) spectra were recorded on a Bruker Vertex 70 FT-IR spectrometer. The NMR spectra were recorded using both a 400 MHz or a 500 MHz Bruker NMR spectrophotometer. Chemical shifts were recorded in parts per million (δ) in CDCl_3 (δ_{H} 7.26 and δ_{C} 77.0 ppm) with TMS as an internal reference. High Resolution Electrospray Ionization Mass Spectra (HRESIMS) were recorded on a Waters XevoTM QToF mass spectrometer in positive ionization mode using the following conditions: 3 kV, 40 V, and 4 V applied to the ESI probe, sample, and extraction cone, respectively; cone and desolvation gas flows of 50 and 300 L/hour, respectively. Spectra were corrected using leucine enkephalin reference peaks from ESI on a 200 pg/ μL solution in 1:1 water:acetonitrile with 0.1% formic acid infused through the LockSprayTM source. Ozone-Induced Dissociation (OzID) mass spectrometry was performed on a modified Thermo Fisher Scientific Orbitrap Fusion mass spectrometer. Thin-layer chromatography (TLC) was performed on silica gel 60 GF₂₅₄ (Merck). Column chromatography (CC) was carried out on silica gel (Merck) type 100 (62–400 μm) and type 60 (5–40 μm) for quick column chromatography (QCC), and Sephadex LH-20 with MeOH elution. All solvents for extraction and chromatography were distilled or purchased in high purities prior to use.

4.2. Plant material

The leaves of *Uvaria cherrevensis* were collected from Doi Suthep National Park, Chiang Mai, Thailand in August 2015. This plant was identified by Dr. Tanawat Chaowasku, CMUB Herbarium, Chiang Mai University, Thailand, where a voucher specimen has been deposited (specimen no. T.Ritthiwigrom 5).

4.3. Extraction and isolation

The leaves of *U. cherrevensis* (1.30 kg) were extracted with MeOH (3 × 3 L) and the extracts were evaporated. The residue was extracted with acetone (500 mL) and concentrated to give the crude acetone extract (126.8 g) as a dark brown gum. The extract was separated by Quick Column Chromatography (QCC) using hexanes to acetone to MeOH to give ten fractions (1–10). Fraction 3 (4.33 g) was subjected to Reversed Phase Column Chromatography (RPCC) by elution with MeOH to give three subfractions (3A–3C). Subfraction 3C (282.3 mg) was separated by CC over silica gel by elution with EtOAc/hexanes (1:4) to provide five subfractions (3C1–3C5). Compound **4** (39.2 mg) as a white amorphous solid, was obtained from subfraction 3C2 after evaporation. Subfraction 3C3 (31.0 mg) was purified after CC over silica gel by elution with MeOH/CH₂Cl₂ (1:99) to provide compound **3** (24.8 mg) as a colourless oil. Subfraction 3C4 (61.1 mg) was isolated by Sephadex LH-20 CC after elution with MeOH to give three subfractions (3C4A–

3C4C). Subfraction 3C4B (25.9 mg) was isolated by CC over silica gel by elution with MeOH/CH₂Cl₂ (1:99) to give compound **1** (7.2 mg) and **2** (10.7 mg) as a white amorphous solid and colourless oil, respectively.

4.3.1. Cherrevenol M (**1**)

White amorphous solid, mp. 70–71 °C; [α]_D²⁴ –90 (c 0.1, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 207 (2.88), 229 (2.93), and 278 (2.55); ECD (c 3.22 mM, MeOH) $\lambda_{\max}$ ($\Delta\epsilon$); 207 (–1.19) and 229 (0.04); IR (neat) $\nu_{\max}$ 3454, 1722, 1276, and 1248 cm^{–1}; ¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 125 MHz) data, see Table 1; HRESIMS m/z 595.3615 [M + Na]⁺ (calcd for C₃₄H₅₂O₇Na, 595.3611).

4.3.2. Cherrevenol N (**2**)

Colourless oil; [α]_D²⁵ –57 (c 0.1, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 203 (4.17), 230 (3.96), and 275 (3.59); ECD (c 0.33 mM, MeOH) $\lambda_{\max}$ ($\Delta\epsilon$); 203 (–72.1) and 230 (–41.3); IR (neat) $\nu_{\max}$ 3437, 1723, 1274, and 1233 cm^{–1}; ¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100 MHz) data, see Table 2; HRESIMS m/z 701.4019 [M + Na]⁺ (calcd for C₄₁H₅₈O₈Na, 701.4029).

4.3.3. Cherrevenol O (**3**)

Colourless oil; [α]_D²⁴ –44 (c 0.1, CHCl₃); UV (MeOH) $\lambda_{\max}$ (log ϵ) 234 (3.61) and 274 (3.34); ECD (c 0.53 mM, MeOH) $\lambda_{\max}$ ($\Delta\epsilon$); 234 (–2.62); IR (neat) $\nu_{\max}$ 3423, 1722, and 1272 cm^{–1}; ¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 125 MHz) data, see Table 2;

Table 2
¹H (400 MHz) and ¹³C NMR data spectroscopic data of cherrevenols N and O (**2–3**) in CDCl₃.

Position	2 ^a		3 ^b	
	δ_{H} , (J in Hz)	δ_{C} , type	δ_{H} , (J in Hz)	δ_{C} , type
1		75.4	C	135.2
2	4.29, d (3.5)	71.2	CH	67.7
3	5.46, m	72.1	CH	5.50, dd (4.8, 2.4)
4	5.84, dddd (10.2, 3.3, 1.5, 0.8)	127.1	CH	4.31, dd (6.5, 2.4)
5	6.14, ddd (10.2, 2.9, 1.5)	128.9	CH	4.13, dd (6.5, 2.9)
6	4.97, m	78.2	CH	6.09, d (2.9)
7	4.87, d (12.3)	66.5	CH ₂	5.09, d (13.1)
	4.72, d (12.3)			4.88, d (13.1)
1'		129.82	C	129.88
2',6'	7.93, dd (7.8, 1.4)	129.76	CH	8.04, dd (7.8, 1.4)
3',5'	7.40, t (7.8)	128.6	CH	7.42, t (7.8)
4'	7.55, tt (7.8, 1.4)	133.4	CH	7.59, tt (7.8, 1.4)
1''		157.3	C	129.88
2''		127.8	C	8.02, dd (7.6, 1.2)
3''	7.32, dd (7.5, 1.8)	130.5	CH	7.45, t (7.6)
4''	6.99, td (7.5, 1.8)	122.0	CH	7.57, tt (7.6, 1.2)
5''	7.26, td (8.2, 1.8)	129.6	CH	7.45, t (7.6)
6''	6.96, d (8.2)	114.3	CH	8.02, dd (7.6, 1.2)
7''	4.81, d (11.9)	69.0	CH ₂	
	4.28, d (11.9)			
1'''	3.49, dt (9.0, 7.0)	71.3	CH ₂	3.69, dt (9.3, 6.8)
	3.46, dt (9.0, 7.0)			3.63, dt (9.3, 6.8)
2'''	1.56, m	29.6	CH ₂	1.64, m
3'''	1.26, m	26.2	CH ₂	1.29, m
4'''–7''' and 12'''–15'''	1.26, m	29.92, 29.9, 29.7, 29.6, 29.57, 29.47, 29.47, 29.4	CH ₂	1.29, m
8'''	1.99, m	27.4	CH ₂	2.03, q (6.5)
9'''	5.34, m	130.0	CH	5.36, m
10'''	5.34, m	130.1	CH	5.36, m
11'''	1.99, m	27.4	CH ₂	2.03, q (6.5)
16'''	1.26, m	32.1	CH ₂	1.29, m
17'''	1.26, m	22.8	CH ₂	1.29, m
18'''	0.88, t (6.9)	14.3	CH ₃	0.90, t (6.8)
3-OC(O)Me		170.1	C	
3-OC(O)Me	1.95, s	21.0	CH ₃	
3-OC(O)				166.64
7-OC(O)		167.1	C	166.60

^a ¹³C NMR recorded at 100 MHz.

^b ¹³C NMR recorded at 125 MHz.

HRESIMS m/z 657.3770 $[M + Na]^+$ (calcd for $C_{39}H_{54}O_7Na$, 657.3767).

4.4. Ozone Induced Dissociation (OzID) mass spectrometry

OzID was performed using a modified Thermo Fisher Scientific Orbitrap Fusion mass spectrometer. Compounds **1–3** were separately dissolved in MeOH to 100 μ M and then diluted to produce 10 μ M analyte in MeOH with 90 μ M sodium acetate. Infusion via syringe pump at 5 μ L/min through an EASY-Max NG source under the following conditions produced an adequate sodium adduct signal: +4.5 kV spray voltage, values of 4, 5, and 1 for sheath, auxiliary, and sweep gas (nitrogen), respectively, 300 and 30 °C ion transfer tube and vaporizer, respectively. The RF lens was set to 60% to transmit ions through the front of the instrument. This mass spectrometer has recently been modified in a manner similar to that reported recently by Paine et al. [17] Oxygen with 0.5% nitrogen (Coregas, New South Wales, Australia) was supplied at 20 psig and 0.2 Standard liters per through a Titan 30 ozone generator (Absolute Ozone, Alberta, Canada) producing 225 g/Nm³ ozone (10.5% by volume in oxygen) as measured in a MiniHICON analyzer (Teledyne API, California, USA). Gas flow was directed through a destruct catalyst (Teledyne API) before expulsion from the laboratory however, prior to destruction, a portion was split-off through a 25 μ m ID/10 cm length PEEKsil restriction (Trajan Scientific Australia, Victoria, Australia) into a flow of helium determined by a regulator upstream of a 50 μ m ID/10 cm length of PEEKsil (Trajan). Under normal operating conditions e.g. in the absence of ozone, the regulator is set such that the ion gauge reads ca. 6×10^{-5} Torr near the ion trap region. The mixture of ozone, oxygen and helium traveled to the ion trap through a 1.4 m length of grey PEEK tubing (381 μ m ID) which bypasses the helium open split inlet. In an MS [3] experiment sodiated precursor ions were isolated through the quadrupole with a 0.8 Da window and allowed to pass into the HCD cell (zero setting on the collision energy) followed by reisolation in the ion trap (2.0 Da window) for 4 s in the presence of ozone. Mass analysis was then performed in the orbitrap analyzer at a 50,000 resolution setting (specified at m/z 200) and a 10000 AGC target. Spectra reported here represent the average over a 1 min acquisition time. The fluoranthene internal calibrant option was used to improve mass accuracy. We note the ozone generator is interlocked to a model 106-L ambient ozone monitor (2B Technologies, Colorado, USA) for safety such that ozone production ceases at or above 60 ppbv laboratory ozone concentration.

4.5. Computational methods

The 3D structures generated were prepared with ChemDraw 17.0 and Avogadro: an open-source molecular builder and visualization tool, Version 1.2.0.61 [18]. The Density Functional Theory (DFT) used to minimize the three-dimensional structures was calculated with B3LYP/6-31G(d) [19–21] using the Gaussian 09 basis set [22].

4.6. Bioactivity assays

Antimalarial activity against *Plasmodium falciparum* TM4/8.2 (a wild type sensitive strain) and K1CB1 (multidrug resistant strain) was carried out as described in a previous report [6]. The cytotoxicity against normal Vero cells (kidney epithelial cells of African green monkey, *Cercopithecus aethiops*) and KB cells (human mouth epidermal carcinoma cells) were also carried out as previously described [6].

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tet.2019.03.004>.

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